

# Photophysical and photochemical properties of 5,7-dimethoxycoumarin under one- and two-photon excitation

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**ABSTRACT:** The spectroscopic and photochemical properties of 5,7-dimethoxycoumarin (DMC) in acetonitrile (ACN) were investigated at room temperature under one- and two-photon excitation. Two-photon induced [2 + 2] photocycloaddition of DMC was investigated with 650 nm laser excitation (120 fs pulse width). GC and GC–MS analysis confirmed dimer formation, while HPLC analysis allowed specific photodimer identification through comparison with samples prepared via one photon irradiation. Photodimer products formed via two-photon excitation correlated with those formed via UV irradiation. *Syn* head-to-head and *syn* head-to-tail photodimers were formed in 1:3.0 and 1:2.8 ratios under one-photon (broadband UV) and two-photon (650 nm, 120 fs) irradiation in anisole, respectively. The quantum yield for the photoreaction of DMC solution ( $10^{-4}$  M) under broadband UV irradiation was of the order of  $1 \times 10^{-3}$  and increased dramatically to  $1 \times 10^{-2}$  under two-photon excitation. The spectral investigation of the photochemical products of DMC revealed efficient fluorescence emission in the spectral region 290–340 nm, attributed to the *syn*-head-to-tail dimers of DMC. Evidence for an additional manifold for the photochemical reaction of DMC was found, resulting in the production of photochemical products that exhibited low fluorescence emission intensity along with dependences on excitation wavelength. Copyright © 2002 John Wiley & Sons, Ltd.

**KEYWORDS:** 5,7-dimethoxycoumarin; photophysical properties; photochemical properties; one- and two-photon excitation; photodimer; photocyclization

## INTRODUCTION

The photochemical [ $\pi 2s + \pi 2s$ ] cycloaddition reaction of the C=C double bond has been successfully exploited in organic synthesis and a number of significant technological applications. The first [2 + 2] photocycloaddition reaction reported was the formation of carvone camphor on exposure of carvone to sunlight by Ciamician in 1908.<sup>1</sup> Both intermolecular and intramolecular photocycloaddition reactions have received considerable attention over the past several decades, emphasizing the importance of this synthetic transformation. The [2 + 2] photodimerization of cinnamate-derivatized polymers

facilitated the development of early photoresists that helped launch photolithography and the printed circuit board industry.

Coumarin derivatives remain a subject of great interest owing to their variety of potential photobiological and therapeutic applications.<sup>2–5</sup> Photochemical properties of 5,7-dimethoxycoumarin (DMC), such as photodimerization,<sup>2,6</sup> photoaddition,<sup>7,8</sup> photoionization, and photosensitized electron-transfer reactions,<sup>9</sup> have been investigated under one-photon excitation by IR, UV, NMR and mass spectrometry,<sup>3</sup> transient absorption spectroscopy<sup>9</sup> and measurement of singlet oxygen yields.<sup>10</sup> Coumarin derivatives continue to be investigated as photorecording materials<sup>11</sup> and as photolabile protecting groups for single- and two-photon uncaging (release) of biologically active substances in cells and tissues.<sup>12</sup>

Dimers of the photocycloaddition of DMC have been isolated and characterized after irradiation in a number of solvents, including acetonitrile (ACN), and the stereochemical and regiochemical configuration of the dimers was elucidated (Fig. 1).<sup>2</sup> Single photon-induced photodimerization of DMC, generating three different photoisomers (*syn* head-to-head, *syn* head-to-tail and *anti* head-to-tail), has been reported.<sup>2,3</sup> Under irradiation with UV

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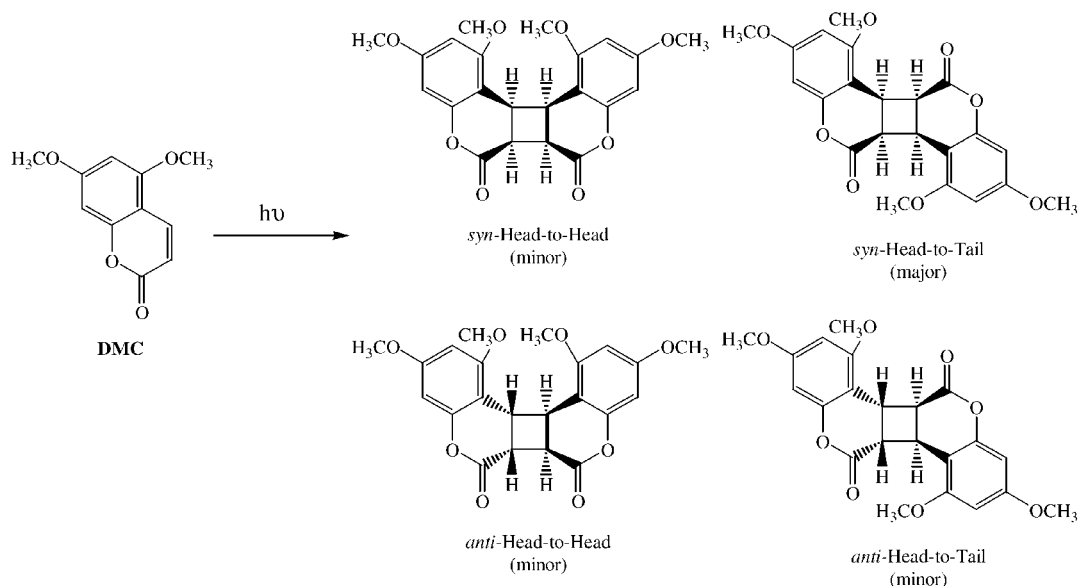
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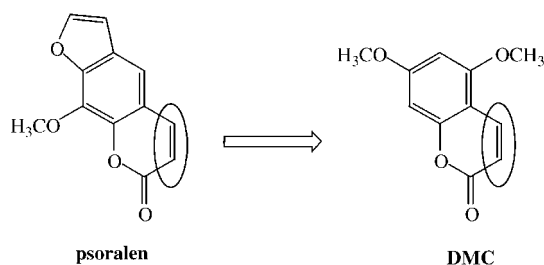
**Figure 1.** Photocycloaddition of DMC

light, psoralens, naturally occurring coumarin derivatives, are known to react with pyrimidine bases in DNA. The [2 + 2] photocycloaddition of DMC has been investigated as a model system for the study of the photoinduced reaction of psoralens and pyrimidine bases in DNA.<sup>13,14</sup>

The photoaddition reaction of DMC with adenosine has been carried out in a dry film state and the structure of the photoproducts was determined by various spectroscopic measurements.<sup>7</sup> The relative photoreactivity of DMC to thymidine in a dry film state was also examined in order to investigate the photoreaction that causes interstrand cross-linking of DNA.<sup>8</sup> The photoionization of DMC was investigated in aqueous solution by transient absorption spectroscopy, and efficient radical cation formation was reported.<sup>9</sup>

However, in previous studies, the photochemical and major spectral parameters of DMC were measured under one-photon excitation without comprehensive optical investigation of its photoproducts. The development of new femtosecond laser sources of irradiation with high

pulse intensity stimulated considerable current interest in research on two-photon photochemical processes. The comparison of the photochemical parameters for one- and two-photon excitation processes is a subject of general importance in coumarin photochemistry, particularly as it pertains to photodynamic therapeutic agent development. DMC is a model for the investigation of two-photon induced photocycloaddition of the enone C=C of psoralens (Fig. 2), an important class of compounds under active study in both one- and two-photon photodynamic therapy.<sup>5,15</sup> Although a wealth of information regarding the understanding and applications of photochemical transformations has been obtained over the last half century, comparatively few studies of multiphoton-induced organic photochemistry have been reported. Therefore, we have conducted a comprehensive investigation of the photophysical and photochemical properties of DMC under both one- and two-photon excitation. The corresponding quantum yields for the photoreactions and their photochemical rate constants were determined.



**Figure 2.** DMC as a model for psoralen enone photochemistry

## EXPERIMENTAL

The spectroscopic and photochemical properties of DMC were measured in ACN at room temperature under one- and two-photon excitation. One-photon absorption spectra were obtained with a Cary-3 UV-visible spectrophotometer. The steady-state fluorescence, excitation and excitation anisotropy spectra were measured in the photon counting regime for dilute solutions of DMC (concentration  $C \leq 6 \times 10^{-6}$  M) in 10 mm quartz cuvettes with a Photon Technologies Inc. (PTI) QuantaMaster

spectrofluorimeter. Excitation anisotropy spectra were measured in a T-format method,<sup>16–19</sup> with baseline correction in viscous silicon oil (viscosity  $\sim 200$  cP at 25 °C) and in ACN at room temperature. The power of the Xe lamp at 230 nm is nearly 30–50 times lower than its maximum power in the spectral range 400–600 nm (Ref. 16, p. 28). Thus, reasonable slit sizes were chosen for the excitation and emission monochromators to obtain a value of the fluorescence intensity of DMC  $\sim 1000$  counts  $s^{-1}$  for excitation wavelengths in the range 230–250 nm. By taking into account the dark noise of the PMT ( $\sim 20$  counts  $s^{-1}$ ), these measurements were sufficiently corrected. The fluorescence lifetime of DMC was obtained with a PTI TimeMaster spectrofluorimeter with excitation using a GL 3300 picosecond-pulsed nitrogen laser (excitation wavelength  $\lambda_{exc} = 337$  nm; pulse duration 600 ps; repetition rate  $f = 10$  Hz). The fluorescence quantum yield of DMC was measured by a standard method relative to 9,10-diphenylanthracene in cyclohexanone at 25 °C.<sup>16</sup> The two-photon absorptivity of DMC was measured by an upconverted fluorescence method,<sup>20</sup> relative to fluorescein in water, with the excitation by Clark-MXR 2001 Ti:sapphire amplified, second harmonic of an erbium-doped fiber ring oscillator system (output 775 nm) that pumped two optical parametric generator/amplifiers (TOPAS, Light Conversion), providing 100–120 fs [full width half-maximum (FWHM)] pulses at a 1 kHz repetition rate with independently tunable wavelengths from 560 to 2100 nm.

Photochemical products of DMC were obtained in ACN solution ( $C \approx 10^{-4}$  M) by one-photon UV lamp irradiation (UVGL-25 UV lamp;  $\lambda_{max} \approx 355$  nm; FWHM  $\approx 15$  nm; irradiation intensity at the exit surface of the lamp  $P \approx 3.5$  mW  $cm^{-2}$ ) in a  $10 \times 10 \times 35$  mm quartz cuvette at room temperature. Photochemical products of DMC under two-photon absorption (2PA) were obtained in a 10  $\mu$ l quartz cuvette by irradiation with the femtosecond laser described above (average irradiation intensity  $P = 90$  mW  $cm^{-2}$ ; excitation wavelength  $\lambda_{exc} \approx 650$  nm).

In order to reveal the nature of the photochemical reactions of DMC, the quantum yields of the photoreactions,  $\Phi$ , for different concentrations of DMC was determined. Under one-photon excitation and for low-concentration solutions, a fluorescence method based on temporal measurements of the fluorescence intensity of DMC was employed. For high-concentration solutions of DMC, an absorption method based on temporal measurements of the absorption spectra of DMC during irradiation was used. The fluorescence method at low concentrations possesses higher accuracy in the determination of  $\Phi$  in comparison with the absorption method, which is more accurate at high concentrations. Under two-photon excitation the same fluorescence method was utilized, owing to the low efficiency of two-photon absorption processes. These methods are described in more detail below.

## One-photon excitation fluorescence method

The general determination of the quantum yield of the photoreaction,  $\Phi$ , is<sup>21</sup>

$$\Phi = N/Q \quad (1)$$

where  $N$  and  $Q$  are the number of molecules that underwent photochemical reaction and the number of absorbed photons, respectively. The value of  $\Phi$  for one-photon excitation at low DMC concentration was determined with the PTI QuantaMaster spectrofluorimeter by measurement of the temporal dependence of the fluorescence intensity of the DMC solution in a  $0.1 \times 0.1 \times 10$  mm microcuvette (Fig. 3). The intensity of the excitation light (Xe lamp of the spectrofluorimeter) was sufficient for photobleaching the DMC solution. The determination of  $\Phi$  was based on the following assumptions:

1. the entire volume of the DMC solution was irradiated in the microcuvette simultaneously, i.e. the effect of diffusion processes on the photochemical kinetics are negligible;
2. the initial optical density,  $D_0(\lambda_{max})$  (optical density at the absorption maximum before irradiation) of the DMC solution in the microcuvette did not exceed 0.1 in the  $z$ -dimension (10 mm pass), i.e. the irradiation intensity,  $I_0$ , was approximately constant in the entire volume of the microcuvette;

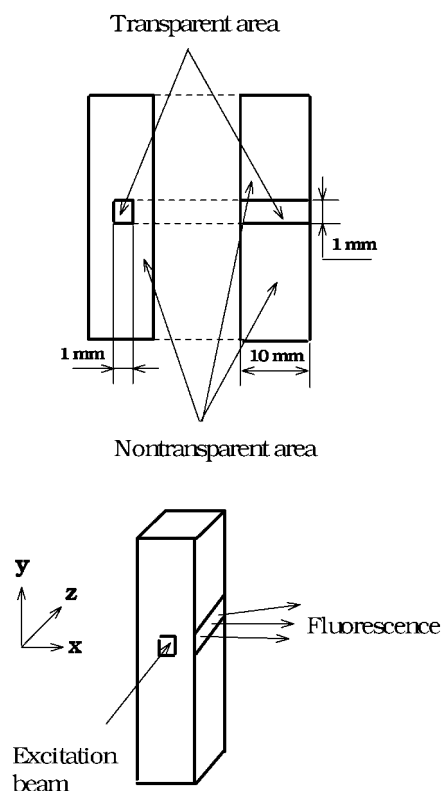


Figure 3. Construction of the microcuvette

3. the optical density and fluorescence of the photochemical products were negligible in the spectral region of the observed fluorescence for DMC, and thus had no effect on the fluorescence intensity of DMC.

Under these conditions, the expression for the quantum yield of the photoreaction under one-photon excitation,  $\Phi$ , is obtained, and is determined by Eqn. (1). The number of photoreacted molecules,  $N$ , can be expressed as

$$N = (C_0 - C_T)V = C_0V(1 - D_T/D_0) \quad (2)$$

where  $C_0$  and  $C_T$  are the initial (before irradiation) and final (after irradiation) concentrations (in molecules  $\text{cm}^{-3}$ ),  $D_0$  and  $D_T$  are initial and final optical densities of the solution and  $V$  is the irradiation volume. The value  $D_T/D_0$  is equal to the ratio of the final,  $F_T$ , and initial,  $F_0$ , fluorescence intensities,  $F_T/F_0$ , based on the assumptions mentioned above. Therefore, Eqn. (2) can be rewritten as

$$N = C_0V(1 - F_T/F_0) \quad (3)$$

The number of photons absorbed during the total irradiation time,  $t = T$ , is

$$Q = \int_0^T Q(t) dt \quad (4)$$

where  $Q(t)$  is the number of photons absorbed per unit time at time  $t$ . The value  $Q(t)$  can be expressed as

$$Q(t) = |dI_0/dz|V = I_0C(t)\sigma(\lambda_{\text{exc}})V \quad (5)$$

where  $I_0$  is the intensity of excitation light,  $C(t)$  is the concentration of molecules in the solution at time  $t$ ,  $\sigma(\lambda_{\text{exc}})$  is the one-photon absorption cross-section at  $\lambda_{\text{exc}}$  and  $z$  is the direction of the excitation. The substitution of Eqn. (5) in Eqn. (4) gives

$$Q = I_0\sigma(\lambda_{\text{exc}})V_0 \int_0^T C(t) dt \quad (6)$$

Substitution of Eqns (6) and (3) in Eqn. (1) yields the expression for the quantum yield of the photoreaction:

$$\Phi = [(1 - F_T/F_0)C_0V]/[I_0\sigma(\lambda_{\text{exc}})V_0 \int_0^T C(t) dt] \quad (7)$$

Taking into account that  $C(t)/C_0 = F(t)/F_0$  [ $F(t)$  is the fluorescence intensity at time  $t$ ], Eqn. (7) can be rewritten as

$$\Phi = (1 - F_T/F_0)/\{I_0 \cdot \sigma(\lambda_{\text{exc}}) \cdot \int_0^T [F(t)/F_0] dt\} \quad (8)$$

where  $F(t)$ ,  $F_T$  and  $F_0$  are expressed in relative arbitrary units (typically in counts  $\text{s}^{-1}$ ),  $I_0$  in photons  $\text{cm}^{-2} \text{s}^{-1}$ ,  $\sigma(\lambda_{\text{exc}})$  in  $\text{cm}^2$  and  $t$  in seconds.

The dependences,  $F(t)$ , and values  $F_T$  and  $F_0$  were measured using a PTI QuantaMaster spectrofluorimeter. The entire volume of the microcuvette was irradiated simultaneously. The intensity of the irradiation light was

calculated as  $I_0 = P_0/S$  ( $P_0$  in photon  $\text{s}^{-1}$ ). We can consider that  $I_0$  is approximately constant in the  $z$ -direction, owing to the low optical density of the investigated solutions [ $D_0(\lambda_{\text{exc}}) \leq 0.1$ ]. The power of the irradiation light,  $P_0$ , which passed through the transverse cross-section,  $S$ , of microcuvette in the  $z$ -direction (see Fig. 3) was measured using a power meter (Laserstar from Ophir Optonics) with sensitivity in the nanowatts regime (i.e. the power in the microwatts range can easily be accurately measured). Thus,  $\Phi$  can be calculated from the temporal fluorescence dependencies,  $F(t)$ , using Eqn. (8).

### One-photon excitation absorption method

The quantum yield of the photoreaction for high-concentration solutions was determined by temporal measurements of the optical density,  $D(t)$ , of the solution in the  $10 \times 10 \times 35$  mm quartz cuvette, during irradiation with a UV lamp (UVGL-25). In this case, the number of molecules that underwent photochemical reaction can be obtained from Eqn. (2) and expressed as

$$N = (C_0 - C_T)V = [D_0(\lambda) - D(\lambda, t)]V/[\varepsilon(\lambda)a] \quad (9)$$

where  $D_0(\lambda)$  and  $\varepsilon(\lambda)$  are the initial optical density of the solution and extinction coefficient of DMC in ACN at the excitation wavelength, respectively, and  $a = 10$  mm (length of the cuvette). The number of absorbed photons of corresponding wavelength,  $\lambda$ , at time  $t$  is

$$Q(\lambda, t) = I_0(\lambda)[1 - 10^{-D(\lambda, t)}]ah \quad (10)$$

where  $10^{-D(\lambda, t)}$  is the transmittance of DMC solution,  $a$  and  $h$  are the dimensions of the irradiated surface of the cuvette ( $a = 10$  mm and  $h = 35$  mm) and  $I_0(\lambda)$  is the spectral distribution of the UV lamp intensity. The total number of photons absorbed from the UV lamp during the irradiation time,  $t = T$ , is

$$Q = \int_0^T \int_0^\lambda Q(\lambda, t) d\lambda dt \quad (11)$$

Substitution of Eqns (9) and (11) in Eqn. (1) gives the expression for the quantum yield of the photoreaction:

$$\Phi = [D_0(\lambda) - D(\lambda, T)]/\{\varepsilon(\lambda)\lambda \int_0^T I_0(\lambda)[1 - 10^{-D(\lambda, t)}] d\lambda dt\} \quad (12)$$

Equation (12) can describe the initial part of the temporal dependence,  $D(\lambda, t)$ , which is characterized by the small change in the optical density [i.e.  $D(\lambda, t)/D_0(\lambda) \approx 1$ ]. In this case, the quantum yield of the photoreaction is

$$\Phi \approx [D_0(\lambda) - D(\lambda, T)]/\{T\varepsilon(\lambda)\lambda \int_0^\lambda I_0(\lambda)[1 - 10^{-D_0(\lambda)}] d\lambda\} \quad (13)$$

where  $[D_0(\lambda) - D(\lambda, T)]/T$  is the initial slope of the

dependence  $D(\lambda, t)$ . Hence Eqn. (13) facilitates the determination of the value of  $\Phi$  for DMC solutions with  $C \geq 10^{-4}$  M (high concentrations).

### Two-photon excitation fluorescence method

The quantum yield of the photochemical reaction under two-photon excitation,  $\Phi_{\text{TPA}}$ , was also determined using the PTI QuantaMaster spectrofluorimeter by excitation using a femtosecond laser (excitation wavelength 600–720 nm; average excitation power,  $P_0$ , up to 1.5 mW; pulse duration 120 fs; repetition rate 1 kHz). The concentration of the DMC molecules in this case was  $10^{-4}$  M (optical density at the absorption band maximum  $\approx 1.5$ ). The intensity of the irradiation,  $I_0$ , was approximately constant over the entire irradiated volume (the same microcuvette depicted in Fig. 3 was employed) owing to the low efficiency of the two-photon absorption processes and absolute transparency of the solution for one-photon absorption in the spectral range 600–720 nm. Hence it can be assumed that:

1. the entire volume of the DMC solution was irradiated simultaneously and  $I_0$  was approximately constant in the entire volume of the microcuvette;
2. the reabsorption of the fluorescence emission in the microcuvette in the  $x$ -direction (1 mm pass) was negligible owing to the low initial optical density of DMC at the absorption maximum [ $D(\lambda_{\text{max}}) \approx 0.15$ ] in this direction;
3. the optical density and fluorescence of the photochemical products of DMC were negligible in the spectral region of the observed fluorescence (for DMC).

Under these conditions, an equation for the two-photon quantum yield,  $\Phi_{\text{TPA}}$ , can be derived. The number of photobleached molecules,  $N$ , in this case is determined from Eqn. (3). The number of absorbed photons per excitation pulse will be equal to twice the number of the molecules excited to the first electronic excited state,  $S_1$ , during one pulse of the irradiation light. In order to calculate this value (number of molecules), balanced equations for two electronic levels of the molecule,  $S_0$  (ground state) and  $S_1$  (first excited state), with corresponding concentrations  $C'_0$  and  $C'_1$ , can be expressed:

$$\begin{aligned} dC'_0(t)/dt &= -C'_0(t)\sigma_{\text{TPA}}I^2(t) & (14) \\ C'_0(t) + C'_1(t) &= C_0 \end{aligned}$$

where  $C_0$  is the total concentration of the molecules,  $I(t) = I_0^{\text{max}} \exp[-(t/\tau_p)^2]$  is the temporal Gaussian distribution of the irradiation intensity with corresponding maximum pulse intensity  $I_0^{\text{max}}$  and pulse duration and  $\tau_p = 120$  fs. In general,  $C_0$  is dependent on irradiation time but during one pulse of excitation the change in  $C_0$

was negligible. In Eqn. (14), the lifetime of the first excited electronic level  $\tau \gg \tau_p$  is taken into account, i.e. no electronic relaxation processes occurred from  $S_1$  during one pulse of excitation. The solution for this system Eqn. (14) is

$$C'_1(t) = C_0 \{1 - \exp[-\sigma_{\text{TPA}} \int I^2(t') dt']\}$$

Hence the number of photons absorbed per pulse of the excitation at time  $t$ ,  $Q_p(t)$ , is

$$Q_p(t) = 2C_0(t)V \{1 - \exp[-\sigma_{\text{TPA}} \int_0^t I^2(t') dt']\}$$

where  $V$  is the irradiated volume of the microcuvette. Next, the number of absorbed photons for pulsed excitation during the time  $t = T$  with repetition rate of the pulses,  $f$  (in Hz), can be written as

$$\begin{aligned} Q &= 2C_0(t=0) \int_0^T (F(t)/F_0) dt V f \\ &\times \{1 - \exp[-\sigma_{\text{TPA}} \int_0^T I^2(t) dt]\} & (15) \end{aligned}$$

The relationship between average power of the laser irradiation,  $P_0$ , and the maximum pulse intensity,  $I_0^{\text{max}}$ , is

$$P_0 = I_0^{\text{max}} S f \int_0^{\infty} \exp[-(t/\tau_p)^2] dt & (16)$$

Taking into account Eqn. (16), the substitution of Eqns (3) and (15) in Eqn. (1) affords

$$\begin{aligned} \Phi_{\text{TPA}} &= (1 - F_T/F_0) / \{2f \int_0^T (F(t)/F_0) dt [1 \\ &- \exp(-\sigma_{\text{TPA}} \alpha P_0^2 (Sf\beta)^{-2})]\} & (17) \end{aligned}$$

where  $\alpha = \int_0^{\infty} \exp[-(t/\tau_p)^2] dt$  and  $\beta = \int_0^{\infty} \exp[-(t/\tau_p)^2] dt$ . For  $\tau_p = 120$  fs,  $\alpha \approx 9 \times 10^{-14}$  s and  $\beta \approx 1.28 \times 10^{-13}$  s. Hence, the two-photon quantum yield of the photoreaction,  $\Phi_{\text{TPA}}$ , can be calculated from the temporal dependencies of the fluorescence intensity,  $F(t)$ , using Eqn. (17).

### One-photon irradiation of DMC in anisole

DMC (0.169 M) dissolved in anisole was transferred to a dried Pyrex glass tube and sealed with a rubber septum. The solution was deaerated with  $N_2$  for 10 min and irradiated with a broadband UV (300–400 nm) lamp in a Rayonet photoreactor for 24 h. The crude mixture was purified by flash column chromatography (silica gel, 1:1 hexane–EtOAc). The products were analyzed on a Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector using a HP-1 column (cross-linked methylsiloxane, 12 m  $\times$  0.2 mm i.d., 0.3  $\mu$ m film thickness). The injector and detector temperatures were set at 200 and 250  $^\circ$ C, respectively, with the oven temperature programmed from 200 to 225  $^\circ$ C at 5  $^\circ$ C  $\text{min}^{-1}$ , at a flow-rate of 1 ml  $\text{min}^{-1}$  using He as the carrier gas. The photochemical products were also subjected to GC–MS analysis on a Hewlett-Packard

Model 5890 gas chromatograph coupled with a Hewlett-Packard Model 5973 mass selective detector equipped with an electron ionization source, quadrupole analyzer and electron multiplier as a detector, under the same temperature control as indicated for GC analysis. Additional photodimer product analysis was performed with a Waters HPLC system equipped with a Model 600 solvent-delivery system, a Model 440 UV absorbance detector (254 nm) and a Model U6K universal injector. The column used was a Whatman Partisil 10 column (250 × 10 mm i.d.) using CH<sub>2</sub>Cl<sub>2</sub>-THF (100: 1, v/v) as eluent at a flow-rate of 0.9 ml min<sup>-1</sup> and UV detection at 254 nm.

### Two-photon irradiation of DMC in anisole

Two-photon photocycloaddition was carried out using a Clark MXR Ti:sapphire femtosecond laser system. The laser beam output was tuned to 650 nm and focused into a 10 μl quartz cuvette. DMC (0.169 M) was dissolved in anisole and purged with N<sub>2</sub> for about 10 min before photolysis. The sample was irradiated at different periods with an average laser beam intensity of about 90 mW cm<sup>-2</sup>. After irradiation, crude reaction mixtures were analyzed by GC, GC-MS and HPLC under the same conditions as for the one-photon irradiation experiments.

## RESULTS AND DISCUSSION

One-photon-induced photocycloaddition of DMC was conducted in anisole in order to provide a reference for subsequent analysis of two-photon-induced photoreactions. The one-photon-induced photolysis products were purified by flash column chromatography prior to product analysis, while the two-photon-induced photolysis products were analyzed without separation owing to the small quantity of photodimers formed under the femtosecond irradiation conditions. The gas chromatogram of the two-photon irradiation (650 nm, 120 fs) mixture displayed a peak with a retention time of 21.6 min, corresponding to the component observed at 21.6 min from the one-photon (350 nm) photoexposure, indicating dimer formation of DMC.

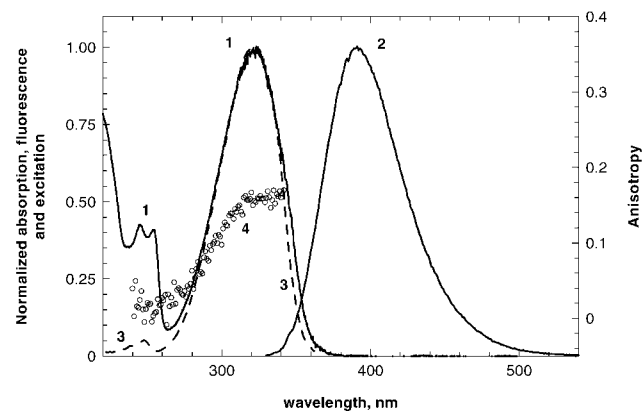
Further photoproduct identification was established through GC-MS analysis. The reconstructed ion chromatogram from one-photon photolysis displayed a dominant peak eluting at 27.7 min. The mass spectrum of that component was in good agreement with literature values,<sup>2</sup> with the molecular ion peak of *m/z* 412 corresponding to DMC dimers. A base peak of *m/z* 206 indicated C<sub>4</sub>-cyclo-dimer fragmentation to yield DMC, while *m/z* 178 and 163 peaks from CO and COCH<sub>3</sub> elimination from DMC were also observed. The two-photon-induced photoproducts were subjected to the same GC-MS conditions as used for one-photon photo-

product analysis. The major component with the retention time of 27.6 min displayed a similar reconstructed ion chromatogram to that observed upon UV exposure, with the dimer molecular ions observed at *m/z* 412, along with the same base and major cleavage fragmentation peaks.

Specific photodimer identification was secured by first comparing the HPLC results of the one-photon photolysis products with literature values.<sup>2,22</sup> Product resolution indicated *syn* head-to-head (H-H) and *syn* head-to-tail (H-T) photodimers were formed in anisole on UV irradiation in a 1:3 ratio, respectively. Likewise, HPLC-resolved photoproducts from two-photon photolysis were comparable to those observed under one-photon conditions, with *syn* H-H identified at 12 min and *syn* H-T at 19.5 min in a 1:2.8 ratio, respectively, nearly the same as that obtained from one-photon UV exposure. Furthermore, the UV-visible absorption spectrum of eluting components displayed photodimer products with an absorption peak ( $\lambda_{\max} \approx 225$  nm) blue shifted relative to DMC ( $\lambda_{\max} \approx 330$  nm).

### Spectroscopic characterization of DMC

The base spectroscopic and photochemical parameters of DMC were measured in ACN at room temperature. The absorption spectrum of DMC in ACN (Fig. 4, curve 1) is characterized by a major absorption peak with  $\lambda_{\max}^a \approx 324$  nm, full width at half-maximum (FWHM)  $\Delta\lambda_{\max}^a \approx 60$  nm, and an extinction coefficient (molar absorptivity)  $\epsilon^{\max} \approx 15 \times 10^3$  M<sup>-1</sup>cm<sup>-1</sup>. Shorter wavelength absorption bands in the spectral region 240–260 nm are associated with transitions to higher excited electronic levels of DMC, demonstrated by the decrease in the excitation anisotropy, *r*, of DMC in viscous silicone oil (Fig. 4, curve 4) in the spectral region 240–260 nm. The maximum value of *r* did not exceed 0.16, even in silicone oil (viscosity  $\sim 200$  cP at 25 °C), in which



**Figure 4.** Normalized absorption (1), fluorescence (2) and excitation (3) spectra of DMC in ACN; excitation anisotropy spectrum (4) of DMC in silicone oil

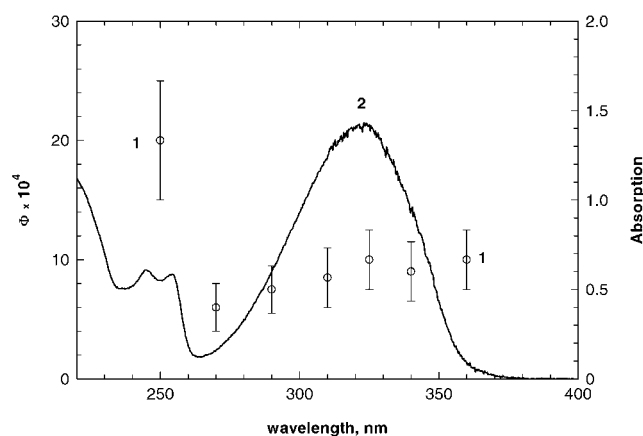
the rotation correlation time,<sup>16,18</sup>  $\tau_R$ , of the DMC molecules essentially exceeds the lifetime of its first excited state,  $\tau$ . Hence all molecules emit before their rotation occurs.

The fluorescence spectrum of DMC in ACN (Fig. 4, curve 2) was independent of the excitation wavelength,  $\lambda_{exc}$ , and characterized by a large Stokes shift ( $\sim 65$  nm). The fluorescence quantum yield,  $\Phi_F$ , of DMC in ACN was  $\sim 0.1 \pm 0.02$ . The excitation spectrum of DMC (Fig. 4, curve 3) was independent of the observed wavelength,  $\lambda_{obs}$ , and corresponded to the absorption spectrum of DMC in the spectral region 280–390 nm (main absorption band). For  $\lambda_{exc} < 280$  nm, a decrease in the excitation spectrum was observed relative to the short-wavelength absorption bands of DMC. The exact reason for the difference between the excitation and absorption spectra in the short-wavelength region is not fully known and warrants further investigation. The fluorescence decay of DMC was measured upon excitation with an N<sub>2</sub> laser ( $\lambda_{exc} = 337$  nm) using the PTI TimeMaster spectrofluorimeter with time resolution  $\sim 0.1$  ns. This decay corresponded to a single-exponential process with lifetime  $\tau \approx 0.85 \pm 0.1$  ns and goodness-of-fit parameter  $\chi^2 \approx 1.05$ .

### Photochemical parameters of DMC

The quantitative determination of the quantum yield of the photochemical reaction,  $\Phi$ , for DMC in ACN was made using the fluorescence method described above. The temporal dependences of the fluorescence intensity,  $F(t)$ , from the DMC solution ( $C \approx 6 \times 10^{-6}$  M) were measured for different  $\lambda_{exc}$ , and  $\Phi$  was calculated from these data using Eqn. (8). The wavelength dependence  $\Phi(\lambda_{exc})$  is presented in Fig. 5, curve 1. From Fig. 5, it can be seen that the value of  $\Phi(\lambda_{exc})$  for DMC in ACN is  $(8 \pm 2) \times 10^{-4}$  and nearly independent of the excitation wavelength in the spectral range  $\lambda_{exc} = 270$ –360 nm. An increase in  $\Phi$  (more than double) was observed for short-wavelength excitation ( $\lambda_{exc} = 250$  nm). This suggests that photochemical reaction from the second electronic excited state ( $S_2$ ) of DMC is more efficient than that from  $S_1$ .

In order to understand more fully the nature of the photochemical processes, the quantum yields of the photoreactions were measured for different concentrations of DMC. For a concentration  $C \approx 10^{-4}$  M, the value of  $\Phi$  was determined by the absorption method described above. The results of the temporal measurements of the absorption spectra of DMC during irradiation are presented in Fig. 6. The quantum efficiency of the photoreaction, determined by Eqn. (13), is  $\Phi \approx 2 \times 10^{-3}$ . Hence  $\Phi$  is dependent on concentration, suggesting that the photochemical processes for DMC solution in ACN are relatively complex. In general, all of the photochemical processes investigated here are first- and

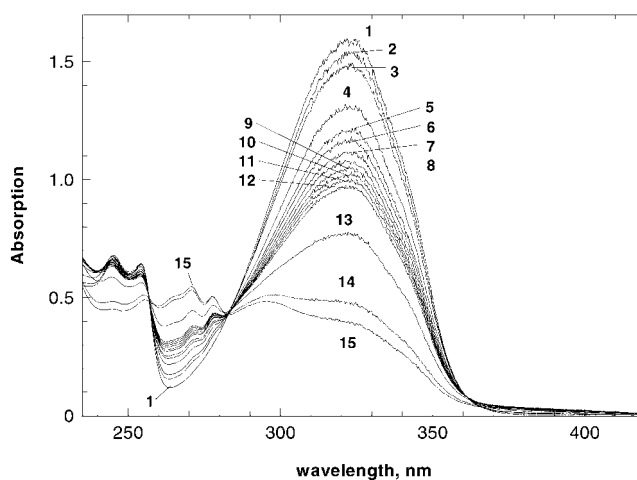


**Figure 5.** Wavelength dependence of the photochemical quantum yield,  $\Phi(\lambda_{exc})$  for DMC (concentration  $6 \times 10^{-6}$  M) in ACN (1); absorption spectrum of DMC in ACN (2)

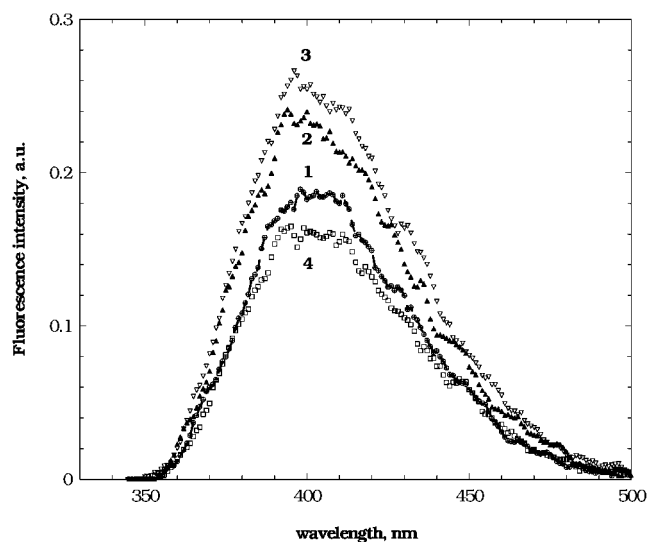
second-order reactions only because the probability of the simultaneous collision of three or more molecules is negligibly small. In this case, the quantum yield of the photoreaction can be written as<sup>20</sup>

$$\Phi = (k_1 + k_2C)/(k_1 + k_2C + 1/\tau) \quad (18)$$

where  $k_1$  ( $s^{-1}$ ) and  $k_2$  ( $M^{-1} s^{-1}$ ) are the photochemical rate constants for first- and the second-order reactions, respectively. Different values of  $\Phi$  at various DMC concentrations,  $\Phi \approx 8 \times 10^{-4}$  at  $C \approx 6 \times 10^{-6}$  M and  $\Phi \approx 2 \times 10^{-3}$  at  $C \approx 10^{-4}$  M, provide an estimate of the photochemical rate constants  $k_1$  and  $k_2$  using Eqn. (18):  $k_1 \approx 8.5 \times 10^5 s^{-1}$  and  $k_2 \approx 1.5 \times 10^{10} M^{-1} s^{-1}$ . According to these data, DMC undergoes different photochemi-



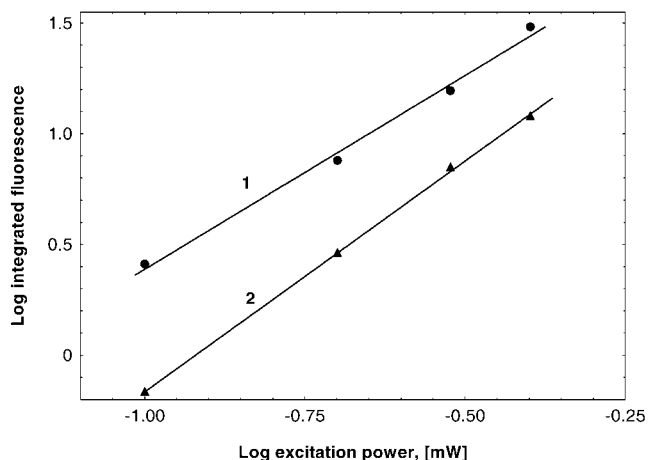
**Figure 6.** Kinetic change in the absorption spectrum of DMC in ACN under irradiation by a UV lamp ( $\lambda_{exc} = 355$  nm; excitation intensity  $I_0 = 3.5$  mW cm<sup>-2</sup>; concentration of DMC =  $10^{-4}$  M): 1, 0 min; 2, 40 min; 3, 80 min; 4, 4 h; 5, 5 h; 6, 6 h; 7, 7 h; 8, 8 h; 9, 9 h; 10, 10 h; 11, 11 h; 12, 12 h; 13, 23 h; 14, 33 h; 15, 42 h



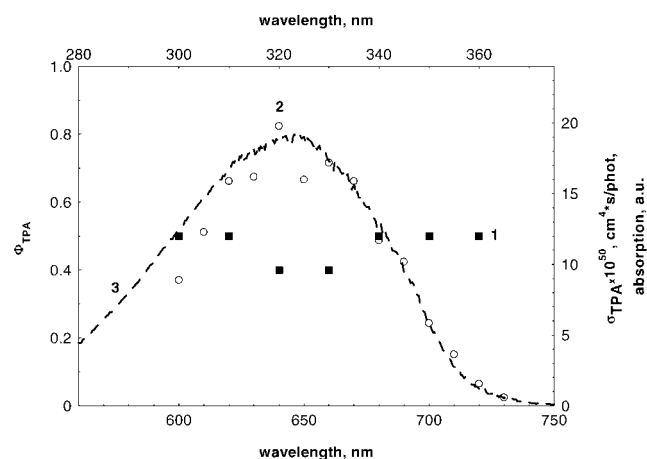
**Figure 7.** Fluorescence spectra of DMC in ACN under the femtosecond two-photon excitation:  $\lambda_{\text{exc}} = 1, 610 \text{ nm}$ ; 2, 630 nm; 3, 640 nm; 4, 680 nm

cal reactions. The first-order photochemical reaction is predominant ( $k_1 \gg k_2C$ ) for concentrations  $C \leq 6 \times 10^{-6} \text{ M}$ . Further investigations are needed to reveal the exact mechanisms. At higher concentrations ( $C \geq 10^{-4} \text{ M}$ ),  $k_1 < k_2C$  and the second-order reaction process dominates. This process can be assigned to dimerization of DMC, which effectively occurs under UV irradiation.<sup>2,3</sup>

The quantum yield of the photoreaction of DMC upon two-photon excitation,  $\Phi_{\text{TPA}}$ , was determined by the fluorescence method described above. The two-photon upconverted fluorescence spectra of DMC were measured (Fig. 7). Comparison of the fluorescence spectra under one- and two-photon excitation (Fig. 4, curve 2 and Fig. 7, respectively) demonstrates that they



**Figure 8.** Dependences of the integrated upconverted fluorescence intensity of DMC in ACN on the excitation power: excitation wavelength = 1, 640 nm (slope 1.8); 2, 700 nm (slope 2.08)



**Figure 9.** Spectral dependence of  $\Phi_{\text{TPA}}(\lambda_{\text{exc}})$ , for DMC in ACN (total accuracy  $\pm 60\%$ ) under two-photon irradiation (curve 1, bottom scale); absorption spectra of DMC in ACN, one-photon excitation (curve 3, top scale) and two-photon excitation (curve 2, bottom scale)

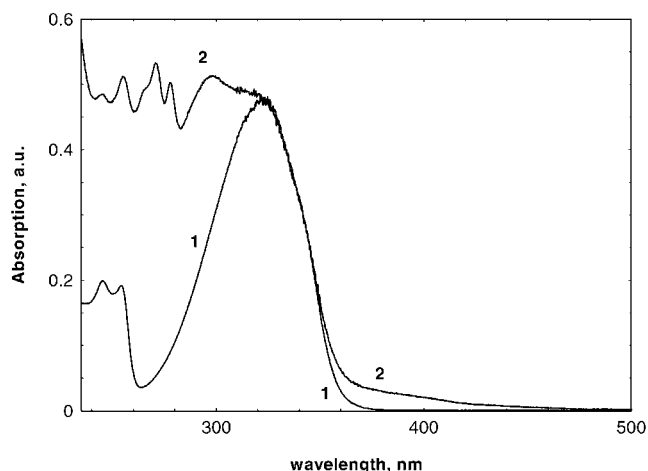
are independent of the type of excitation (one- vs two-photon). The fluorescence intensity under two-photon excitation exhibited a quadratic dependence on the irradiation power (Fig. 8), characteristic of a simultaneous two-photon absorption (TPA) process.

The TPA cross-section of DMC in ACN was measured (Fig. 9, curve 2) by the upconverted fluorescence method,<sup>20</sup> relative to fluorescein in water. The temporal dependences,  $F(t)$ , of the upconverted fluorescence of DMC solution were then measured in a microcuvette under femtosecond laser excitation, and the value of  $\Phi_{\text{TPA}} \approx 1 \times 10^{-2}$  was calculated from Eqn. (17). The total accuracy of the determination of  $\Phi_{\text{TPA}}$  was ca  $\pm 60\%$  owing to the large error in the determination of the TPA cross-section. The spectral dependence  $\Phi_{\text{TPA}}(\lambda_{\text{exc}})$  is presented in Fig. 9, curve 1. From these data, it follows that the value of  $\Phi_{\text{TPA}}$ , within experimental limits, is significantly larger than the corresponding value of  $\Phi$  for one-photon excitation ( $\Phi \approx 1 \times 10^{-3}$ ). This result can be explained by the general theory of the two-photon absorption process, which involves transitions to higher energy electronic excited states that exhibit correspondingly higher photochemical reactivity. Another possible explanation involves weak, additional one-photon reabsorption processes from the first excited state  $S_1$  to higher energy excited states (more reactive), which do not change the population of  $S_1$  and, therefore cannot be reflected in the linear dependence in Fig. 8. Further investigations are needed to understand the exact reason of this photochemical behavior.

### Photochemical products of DMC

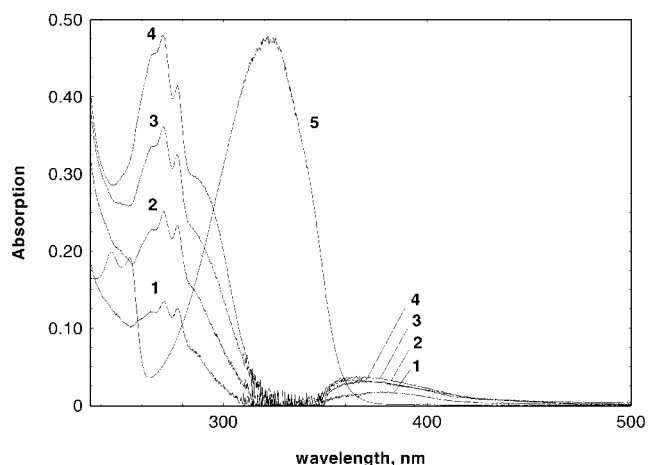
The photochemical products of DMC in ACN were obtained after irradiation with a UV lamp (UVGL-25).



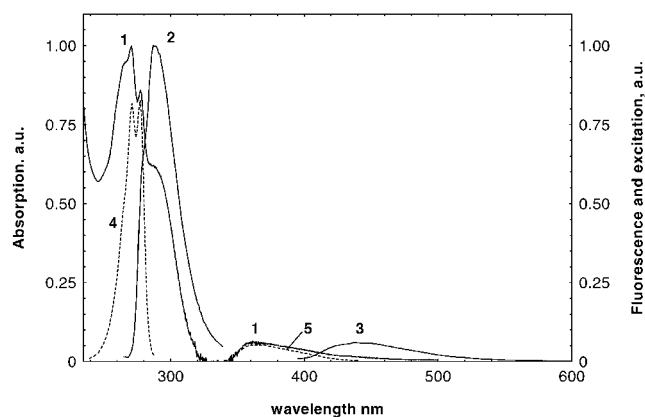


**Figure 10.** Normalized (at 340 nm) absorption spectra of the DMC in ACN before (1) and after 42 h (2) of irradiation by a UV lamp (UVGL-25; excitation wavelength 355 nm; excitation intensity  $I_0 = 3.5 \text{ mW cm}^{-2}$ )

The irradiation intensity at the front surface of the cuvette was  $\sim 3.5 \text{ mW cm}^{-2}$  and the concentration of the DMC solution was  $\sim 10^{-4} \text{ M}$ . Time-dependent changes in absorption spectrum of DMC in ACN, under these irradiation conditions, are presented in Fig. 6. It can be seen that new absorption bands appeared in the spectral regions 260–290 and 380–420 nm, corresponding to photochemical products of DMC. The shape of the absorption spectrum of the DMC solution after irradiation (curve 15) corresponded precisely to the shape of the absorption spectrum of pure DMC before irradiation (curve 1) in the spectral region 330–350 nm. In Fig. 10, the normalized (over the spectral region 330–350 nm) curves (1 and 15 from Fig. 6) are presented (curves 1 and 2, respectively). This means that the optical density of the



**Figure 11.** Extracted absorption spectra of the photochemical products of DMC in ACN after various irradiation times: 1, 4 h; 2, 10 h; 3, 23 h; 4, 33 h; 5, absorption spectrum of DMC before irradiation



**Figure 12.** Absorption spectrum (1), fluorescence spectra [excitation wavelength 270 nm (2) and 390 nm (3)] and excitation spectra [observed wavelength 295 nm (4) and 460 nm (5)] of the photochemical products of DMC in ACN

photochemical products of DMC in this spectral region is negligible, making it possible to extract the real absorption spectrum of the photochemical products from the total absorption spectrum (curve 2). The extracted absorption spectra of the photochemical products of DMC, for different irradiation times, are presented in Fig. 11. Evident is a slight change in the spectral shape of the absorption spectrum of the photochemical products during irradiation.

The fluorescence and excitation spectra of the photochemical products are presented in Fig. 12. The main photochemical products of DMC in ACN upon excitation in the main absorption band were assigned to *syn* head-to-tail dimers.<sup>3</sup> This was also demonstrated by the isolation and characterization of these dimers as described above. The absorption maxima of *syn* head-to-tail dimers occur at  $\sim 280 \text{ nm}$ , which corresponds approximately to the excitation spectrum (curve 4) in Fig. 12. The short-wavelength fluorescence band (Fig. 12, curve 2) was independent of the excitation wavelength. Therefore, this fluorescence emission probably belongs to *syn* head-to-tail dimers, the most probable photoproducts of DMC formed in polar solvents such as ACN. Likewise, the short-wavelength part of the absorption spectrum (Fig. 12, curve 1) probably belongs to other DMC dimers (e.g. *syn* head-to-head and/or *anti* head-to-head)<sup>3</sup> or, possibly, other photochemical products. The intensities of the fluorescence and excitation bands in the spectral region  $\lambda \geq 350 \text{ nm}$  were more than 10 times weaker and exhibited dependences on the excitation and observed wavelength, respectively. Therefore, in addition to photodimers, another manifold(s) for formation of photochemical products of DMC exists under the irradiation (wavelength-dependent) conditions (e.g. photoionization to form stable cation radicals).<sup>9</sup> Although reports of wavelength-dependent photochemical quantum yields are rare, they have appeared, e.g., measurements of the photochemical quantum yields,  $QY$ , were reported for

Rhodamine 6G under excitation at 546 nm ( $QY \approx 10^{-7}$ ), 365 nm ( $QY \approx 10^{-5}$ ) and 313 nm ( $QY \approx 10^{-2}$ ).<sup>23</sup>

## CONCLUSIONS

The two-photon induced [2 + 2] photocycloaddition of DMC was investigated at 650 nm. GC-MS analysis confirmed dimer formation, and HPLC analysis allowed specific photodimer identification through comparison with samples prepared via one-photon UV exposure. Photodimer products formed via two-photon excitation correlated, in nearly the same ratios, with those formed with UV irradiation. A quadratic dependence as a function of incident intensity (at 650 and 700 nm) was observed through the two-photon upconverted fluorescence spectra of DMC, confirming the two-photon nature of excitation under these conditions.

The main spectroscopic and photochemical parameters of DMC in ACN were investigated under one- and two-photon excitation. The quantum yields of the photochemical reactions of DMC were independent of the excitation wavelength in the main absorption band. The values of  $\Phi$  were  $(8 \pm 2) \times 10^{-4}$  and  $(2 \pm 0.5) \times 10^{-3}$  for DMC (concentrations  $6 \times 10^{-6}$  and  $10^{-4}$  M, respectively) under one-photon excitation. For low-concentration solutions ( $C \leq 6 \times 10^{-6}$  M), a first-order photochemical reaction of DMC was observed with a corresponding rate constant  $k_1 \approx 8.5 \times 10^5 \text{ s}^{-1}$ . At higher concentrations ( $C \geq 10^{-4}$  M), a second-order photoreaction of DMC was dominant, with the photochemical rate constant  $k_2 \approx 1.5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ . The quantum yield of the photoreaction of DMC under two-photon excitation,  $\Phi_{\text{TPA}}$ , increased dramatically up to  $\Phi_{\text{TPA}} \approx 1 \times 10^{-2}$  at a concentration  $C \approx 10^{-4}$  M, possibly owing to transitions to higher energy electronic excited states that exhibit correspondingly higher photochemical reactivity and/or weak, additional one-photon reabsorption processes from the first excited state  $S_1$  to higher energy excited states that possess higher reactivity.

The spectral investigation of the photochemical products of DMC revealed an efficient fluorescence band in the spectral region 290–340 nm, which was independent of excitation wavelength. This band was assigned to the fluorescent *syn* head-to-tail dimers of DMC, which are effectively formed under the excitation of DMC in ACN. Other dimers and photochemical products exhibited low fluorescence intensity and dependences on the excitation wavelength.

The many advantages associated with non-linear absorption-induced processes are fast propelling two-photon absorbing materials and photochemical transformations to the forefront of several important fields.<sup>24</sup> One can anticipate the continued deployment of such materials and processes in 3-D volumetric optical recording, non-destructive 3-D imaging, optical sensor protection, photodynamic therapy and 3-D nano- and

microfabrication. We can expect to witness breakthroughs in years to come, owing to the harnessing of 3-D spatially resolved two-photon-induced photochemical reactions in organic materials. However, the complicated photochemical processes observed for DMC under one- and two-photon excitation underscore the need for further comparative photochemical investigations.

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